

Claims

1. A device for non-invasive measurement of the individual metabolic rate of a substantially spherical metabolizing particle, which device comprises

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a) at least one compartment, said compartment being defined by a diffusion barrier and capable of comprising a medium with a substantially spherical metabolizing particle, said diffusion barrier allowing metabolite transport to and/or from the substantially spherical metabolizing particle by means of diffusion, whereby a metabolite diffusion gradient is allowed to be established from the substantially spherical metabolizing particle and throughout the medium,

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b) at least one detector for measuring the concentration of a metabolite inside the compartment.

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2. The device according to claim 1, wherein the diffusion barrier is constituted by a compartment wall having at least one metabolite permeable opening and the medium.

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3. The device according to claim 2, wherein the compartment wall is produced from a substantially metabolite impermeable material.

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4. The device according to claim 3, wherein the substantially metabolite impermeable material has a metabolite diffusion coefficient less than 1 % of the metabolite diffusion coefficient in water, particularly less than 0.2%, most particularly less than 0.05%.

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5. The device according to any of the claims 2-4, wherein the metabolite flux through the compartment wall of substantially metabolite impermeable material constitutes less than 10 % of the total metabolite flux to the compartment, particularly less than 1%, most particularly less than 0.1%

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6. The device according to claim 3, wherein the substantially gas impermeable material is selected from the group of materials of plastics, polymer material,

glass material, metallic material, and ceramic material as well as combinations thereof.

- 5 7. The device according to claim 6, wherein the polymer material is selected from the group of polymers of acetal resins, acrylic resins, cellulosic plastics, fluoroplastics, ionomers, parylenes, polamides, polyamide nanocomposites, polycarbonates, polyesters, polyimide, polyolefins, polyphenyle sulfides, polysulfones, styrenic resins, vinyl resins, plastic alloys, multiplayer polymers, epoxy resins, olefins thermoplastic elastomers, polyether block amides, polybutadiene thermoplastic elastomers styrenic thermoplastic elastomers, vinyl thermoplastic elastomers, rubber materials such as butadiene rubber, butyl rubber, bromobutyl rubber, chlorobutyl rubber, polyisobutylene rubber, chlorosulfonated polyethylene rubber, epichlorohydrin rubber, ethylene-propylene rubber, fluoroelastomers, natural rubbers, neoprene rubbers, nitrile rubbers, polysulfide rubbers, 10 polyurethane rubbers, silicone rubbers, styrene-butadiene rubbers or copolymers thereof.
- 15 8. The device according to claim 1, wherein the diffusion barrier is constituted by a high-viscosity medium.
- 20 9. The device according to claim 8, wherein the high-viscosity medium is due to a high concentration of organic solutes selected from the group of dextrans, glycerol, sugars, carbohydrates, proteins, and inorganic salts.
- 25 10. The device according to any of the preceding claims, wherein the shape of the compartment is selected from the group of a cylinder, a polyhedron, a cone, a hemisphere or a combination thereof.
- 30 11. The device according to claim 10, wherein the general shape of the compartment is a cylinder.
12. The device according to any of the preceding claims comprising an insert for the adjustment of the transverse dimension of the compartment .

13. The device according to any of the preceding claims, wherein the compartment has an adjustable bottom in order to change the dimensions and either increase or decrease the compartment volume.
- 5 14. The device according to any of the preceding claims, wherein the transverse dimension is less than 2.5 mm, particularly less than 1.5 mm, more particularly less than 500 μm , such as less than 250 μm .
- 10 15. The device according to claim 12, wherein the transverse dimension of the insert is less than 1.5 mm, particularly less than 1.0 mm, more particularly less than 500 μm , even more particularly less than 300 μm .
- 15 16. The device according to any of the preceding claims wherein the longitudinal dimension of the compartment is between 2 mm to 25 mm, particularly between 3 mm to 15 mm.
17. The device according to claim 2, wherein the metabolite permeable opening is constituted by a metabolite permeable membrane.
- 20 18. The device according to claim 17, wherein the metabolite permeable membrane is produced from a material comprising silicone, Teflon fluoropolymers, or plastic compounds such as polyethylene, polypropylene or neoprene.
- 25 19. The device according to claim 17, wherein the metabolite permeable membrane is produced from a material comprising permeable matrixes or porous material such as glass, ceramics, minerals, glass or mineral fibers, or precious metal such as gold or platinum.
- 30 20. The device according to claim 17, wherein the metabolite permeable membrane is produced from a material comprising silicone.
21. The device according to any of the preceding claims, wherein a metabolite permeable layer is arranged in the bottom of the at least one compartment.

22. The device according to claim 21, wherein the metabolite permeable layer is produced from a material comprising silicone, Teflon fluoropolymers, plastic compounds such as polyethylene, polypropylene or neoprene.
- 5 23. The device according to claim 21, wherein the metabolite permeable layer is produced from a material comprising permeable matrixes or porous material such as glass, ceramics, minerals, glass or mineral fibers, or precious metal such as gold or platinum.
- 10 24. The device according to claim 21, wherein the metabolite permeable layer is produced from a material comprising silicone.
- 15 25. The device according to any of the preceding claims 21-24, wherein the thickness of the metabolite permeable layer is at least 100 μm , particularly at least 300 μm , and more particularly at least 900 μm .
- 20 26. The method according to any of the preceding claims, wherein the metabolite detector is placed at the bottom of the compartment.
27. The method according to any of the claims 21-26, wherein a metabolite permeable layer is placed between the substantially spherical metabolizing particle and the metabolite detector.
- 25 28. The method according to any of the claims 21-27, wherein the metabolite permeable layer has a thickness of at least twice the diameter of the substantially spherical metabolizing particle.
29. The device according to any of the preceding claims, wherein the metabolite is a gas.
- 30 30. The device according to any of the preceding claims, wherein the metabolite is oxygen or carbon dioxide.
- 35 31. The device according to any of the preceding claims, wherein the detector is an oxygen detector.

32. The device according to claim 31, wherein the detector for measuring the oxygen concentration comprises amperometric oxygen sensors, membrane inlet mass spectrometry, microspectrophotometry, or optical oxygen sensing.
- 5 33. The device according to claim 32, wherein the optical oxygen sensing is performed using a luminophore, particularly an immobilized luminophore placed inside the compartment, more particularly in the bottom, and a detector of luminescence.
- 10 34. The device according to claim 33, wherein the luminophore comprises Ruthenium(II)-tris-4,7-diphenyl-1,10-phenantroline per chlorate (Rudpp) immobilised in a polystyrene matrix, Ruthenium (II) tris-1,7-diphenyl-1,10-phenanthroline chloride, Ruthenium(II)-tris(bipyridyl) complex, Tris (2,2'-bipyridyl di-chloro-ruthenium) hexahydrate, Ru(bpy), Platinum (II)-octa-ethyl-porphyrin in polystyrene, Platinum (II)-octa-ethyl-porphyrin in poly(methyl-methacrylate), Platinum (II)-octa-ethyl-ketoporphyrin in polystyrene, Platinum (II)-octa-ethyl-ketoporphyrin, Palladium (II)-octa-ethyl-porphyrin in polystyrene, Platinum-1,2-ene-dithiolates class of compounds.
- 15 35. The device according to claim 33, wherein the detector of luminescence is a luminescence reader, a photomultiplier tube or a CCD camera (12).
- 20 36. A non-invasive method for determining the metabolic rate of a substantially spherical metabolizing particle, comprising
- 25 a) providing at least one device as defined in any of claims 1-35,
- b) arranging a substantially spherical metabolizing particle in the medium of a compartment,
- 30 c) measuring a metabolite concentration inside the compartment obtaining a metabolite concentration measure, and
- d) correlating said metabolite concentration measure to a metabolic rate of said substantially spherical metabolizing particle.
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37. The method according to claim 36, wherein metabolite is supplied to the substantially spherical metabolizing particle by diffusion through the medium.
38. The method according to any of the claims 36-37, wherein the substantially spherical metabolizing particle is cultured in the compartment.
39. The method according to any of the claims 36-38, wherein the metabolite concentration is measured in a volume smaller than the volume of the compartment and/or the volume of the medium.
40. The method according to any of the claims 36-39, wherein the metabolic rate of said substantially spherical metabolizing particle is determined by determining a metabolite diffusion gradient in the compartment based on the measured metabolite concentration, and correlating said metabolite diffusion gradient to the metabolic rate of said substantially spherical metabolizing particle.
41. The method according to any of the claims 36-40, wherein at least two measurements of the metabolite concentration are performed.
42. The method according to any of the claims 36-41, wherein the metabolite concentration is a gas partial pressure
43. The method according to claim 42, wherein the gas partial pressure is the partial pressure of oxygen or carbon dioxide.
44. The method according to any of the claims 36-43, wherein gas is supplied to the substantially spherical metabolizing particle by diffusion through the stagnant medium in the compartment directly from the atmosphere or from a larger volume of medium in equilibrium with the atmosphere.
45. The method according to any of the claims 36-44, wherein the substantially spherical metabolizing particle is selected from the group of an embryo, group of cells, such as cancer cell(s), stem cells, embryonal stem cells, *C. elegans* or other small multicellular organisms.

46. The method according to claim 45, wherein the substantially spherical metabolizing particle is an embryo.
47. The method according to any of the preceding claims 36-46, wherein the measurement of the concentration of the metabolite is conducted after a temporary elimination of diffusive metabolite supply to the compartment from outside the compartment.
48. A method for regulating metabolite supply to a substantially spherical metabolizing particle during culturing, comprising
- a) providing at least one device comprising a compartment with a medium,
 - b) culturing a substantially spherical metabolizing particle in the medium of a compartment,
 - c) measuring a metabolite concentration inside the compartment obtaining a metabolite concentration measure, and optionally
 - d) correlating said metabolite concentration measure to a metabolic rate of said substantially spherical metabolizing particle and optionally
 - e) regulating the metabolite supply depending on the metabolite concentration measure and/or the metabolic rate of said substantially spherical metabolizing particle.
49. The method according to claim 48, wherein at least one of the devices is as defined in any of claims 1-35,
50. The method according to claim 48 or 49, wherein the metabolite is a gas.
51. The method according to claim 50, wherein the metabolite is oxygen and the metabolic process is respiration.

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52. The method according to claim 48 or 49, wherein the regulation is conducted by changing the metabolite concentration outside the compartment.
53. The method according to claim 48 or 49, wherein the regulation is conducted by changing the dimensions of the compartment.
54. The method according to claim 53, wherein the volume is adjusted by inserting an insert.
55. The method according to claim 53, wherein the transverse dimensions of the compartment is adjusted by inserting an insert.
56. The method according to claim 53, wherein the volume is adjusted by shifting the position of an adjustable bottom of the compartment.
57. The method according to claim 53, wherein the regulation is conducted by changing the diffusion barrier of the compartment.
58. The method according to claim 53, wherein the diffusion barrier is changed by changing the thickness of a compartment wall.
59. The method according to claim 53, wherein the regulation is conducted by changing the size of at least one opening in the compartment wall.
60. A method for selecting a viable embryo comprising,
- a) determining the metabolic rate of the embryo at least once during culturing ,
and
 - b) selecting the embryo having an optimal metabolic rate.
61. The method according to claim 60, wherein the determination of the metabolic rate is conducted without causing any change in the growth conditions experienced by the embryo.

62. The method according any of the claims 60-61, wherein the metabolic rate is measured in a device as defined by any of the claims 1-35.
63. The method according any of the claims 60-61, wherein the metabolic rate is determined by a method as defined in any of claims 36-47.
64. A non-invasive method for determining the metabolic rate of a metabolizing particle, comprising
- a) providing at least one device as defined in any of claims 1-35,
 - b) culturing a metabolizing particle in the medium of a compartment,
 - c) reducing metabolite supply to the medium during at least a part of the culturing period,
 - d) measuring a metabolite concentration inside the compartment obtaining a metabolite concentration measure after the metabolite supply has been reduced, and
 - e) correlating said metabolite concentration measure to a metabolic rate of said substantially spherical metabolizing particle.
65. The method according to claim 64, wherein the metabolite is oxygen and the metabolic rate is the respiration rate.
66. The method according to claim 64, wherein the oxygen supply is reduced to zero.
67. The method according to claim 64, wherein the gas partial pressure measure in the compartment has been obtained during the period of reduced oxygen supply
68. A culture device for culturing a metabolizing particle, which device comprises at least one compartment, said compartment being defined by a diffusion barrier and capable of comprising a medium with a metabolizing particle, said diffusion barrier allowing metabolite transport to and/or from the metabolizing particle by means of diffusion,

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whereby a metabolite diffusion gradient is allowed to be established from the metabolizing particle and throughout the medium.

5 69. The device according to claim 68, wherein said device has one or more of the features as defined in any of claims 1-35.

70. A method for culturing a metabolizing particle, said method comprising

10 a) providing at least one device as defined in any of claims 68-69,

b) arranging a metabolizing particle in the medium of the compartment,
and

15 c) culturing the metabolizing particle.